Amendments to the Specification

The Cross-Reference To Related Applications section at page 1.

This application is a continuation of U.S. Patent Application Serial No. 09/778,187, filed February 6, 2001, which is a continuation-in-part of pending International Application No. PCT/US99/17905, filed 05 August 1999, which was published under PCT Article 21(2) on 17 February 2000, in English, as WO 00/08158, and which claims the benefit of U.S. Provisional Application Serial No. 60/095,672, filed 07 August 1998, now abandoned. International Application No. PCT/US99/17905 and U.S. Provisional Application Serial No. 60/095,672 are incorporated herein by reference.

Paragraph at page 1, lines 25-34:

Adhesion molecules play important roles in cell signaling within the immune system and other cellular systems. In addition to the antigen specific signals delivered by the T cell receptor complex, the shape and type of immune response by T cells depend upon costimulatory signals mediated by adhesion molecules on antigen presenting cells (APC). One such costimulatory signaling ~~inveles~~ involves the adhesion molecules B7-1 (CD80) and B7-2 (CD86) which send important signals through their T cell surface receptors, CD28 and CTLA4 (CD152). B7-1 interacts with CD28 to signal cytokine production, cell proliferation, and the generation of effector and memory T cells. If the signal through CD28 is blocked, T cell anergy or immune deviation can occur, resulting in severely depressed or altered immune responses.

Paragraph at page 2 line 33 to page 3 line 12:

The present invention provides mammalian polypeptides, designated LDCAM, so designated because they are found on lymphoid derived dendritic cells and display a limited homology to adhesion molecules, including B7-1. The LDCAM molecules described herein include isolated or homogeneous proteins that bind to themselves, have limited homology with B7L-1 (described in copending application S/N 60/095,663 filed August 7, 1998 (incorporated herein by reference) and for which B7-L1 is a binding protein. The present invention further includes isolated DNAs encoding LDCAM and expression vectors comprising DNA encoding mammalian LDCAM. Within the scope of this invention are host cells that have been transfected or transformed with expression vectors that comprise a DNA encoding LDCAM, and processes for producing LDCAM by culturing such host cells under

conditions conducive to expression of LDCAM. Further within the present invention are pharmaceutical ~~composition~~ compositions comprising soluble forms of LDCAM molecules and methods for modulating T cell immune responses by administering the pharmaceutical compositions. Additional methods encompassed by the present invention include generating natural killer cells by administering pharmaceutical compositions to an individuals or by combining LDCAM and natural killer cell precursor cells ex vivo.

Paragraph at page 3, lines 31 to page 4, line 3:

To identify cell lines to which B7L-1 binds and to subsequently isolate a protein to which B7L-1 binds, a B7L-1/Fc fusion protein was prepared as described in Example 1 and binding studies, described in Example 2, were carried out. Example 3 describes screening a cDNA library prepared from WI-26, a cell line to which B7L-1 binds, and identifying a full length LDCAM human clone. The nucleotide sequence encoding human LDCAM, isolated as described in Example 3, is presented in SEQ ID NO:1, and the amino acid sequence encoded thereby is presented in SEQ ID NO:2. The encoded human LDCAM amino acid sequence described in SEQ ID NO:2 has a predicted extracellular domain of 374 amino acids including a leader sequence of 38 amino acids 1-38; a transmembrane domain of 21 amino acids (375-395) and a cytoplasmic domain of 47 amino acids (396-442).

Paragraph at page 4, lines 4-15:

Examples 5 and 6 describe making and using a human LDCAM/Fc in binding studies to identify cell lines to which the human LDCAM binds. Among cell lines positively identified were S49.1 cells and lymphoid dendritic cells from spleens and lymph nodes of Flt3-L treated mice. Example 7 describes screening pools of an expression library to identify murine LDCAM clones. The isolated murine LDCAM DNA sequence is disclosed in SEQ ID NO:3. The amino acid sequence encoded by the nucleotide sequence of SEQ ID NO:3 is disclosed in SEQ ID NO:4. The encoded murine LDCAM amino acid sequence (SEQ ID NO:4) has a predicted extracellular domain of 356 amino acids (residues 1-356); a transmembrane domain of 21 amino acids (357-377); and a cytoplasmic domain that includes amino acid residues 378-423~~)~~. SEQ ID NO:3 and SEQ ID NO:4 describes the full length mature murine LDCAM sequences. As compared to the human LDCAM sequence, the signal sequence is not completely described.

Paragraph at page 5, lines 1-8:

As used herein, the term LDCAM encompasses polypeptides having the amino acid sequence 1-442 of SEQ ID NO:2 and the amino acid sequence 1-423 of SEQ ID NO:4. In addition, LDCAM encompasses polypeptides that have a high degree of similarity or a high degree of identity with the amino acid sequence of SEQ ID NO:2, the amino acid sequence of SEQ ID NO:4, and which polypeptides are biologically active. ~~the~~The term "LDCAM" refers to a genus of polypeptides that bind and complex with themselves, polypeptides for which B7L-1 is a binding protein, and polypeptides that alter T cell signals in response to antigen and mitogens.

Paragraph at page 5, lines 9-17:

The term "murine LDCAM" refers to biologically active gene products of the DNA of SEQ ID NO:3 and the term "human LDCAM" refers to biologically active gene products of the DNA of SEQ ID NO:1. Further encompassed by the term "LDCAM" are soluble or truncated proteins that comprise primarily the B7L-1 co-binding portion of the protein, retain biological activity and are capable of being secreted. Specific examples of such soluble proteins are those comprising the sequence of amino acids 1-374 of SEQ ID NO:2 and those comprising the sequence of amino acids 1-356 of SEQ ID NO:4. Alternatively, such soluble proteins can exclude a leader sequence and thus encompass amino acids 39-374 of SEQ ID NO:2.

Paragraph at page 7, lines 9-24:

Examples of soluble LDCAM polypeptides include those comprising a substantial portion of the extracellular domain of a native LDCAM protein. For example, a soluble human LDCAM protein comprises amino acids -38-374 or 1-374 of SEQ ID NO:2 and a soluble murine LDCAM includes amino acids 1-356 of SEQ ID NO:4. In addition, truncated soluble LDCAM proteins comprising less than the entire extracellular domain are included in the invention. When initially expressed within a host cell, soluble LDCAM may include one of the heterologous signal peptides described below that is functional within the host cells employed. Alternatively, the protein may comprise the native signal peptide. In one embodiment of the invention, soluble LDCAM can be expressed as a fusion protein comprising (from N- to C-terminus) the yeast α-factor signal peptide, a FLAG® peptide described below and in U.S. Patent No. 5,011,912, and soluble LDCAM consisting of amino

acids 39-374 of SEQ ID NO:2 or 21-356 of SEQ ID NO:4. This recombinant fusion protein is expressed in and secreted from yeast cells. The FLAG® peptide facilitates purification of the protein, and subsequently may be cleaved from the soluble LDCAM using bovine mucosal enterokinase. Isolated DNA sequences encoding soluble LDCAM proteins are encompassed by the invention.

Paragraph at page 15, lines 3-22:

NK cells are large granular lymphocytes that are distinct from T or B lymphocytes in morphology and function. NK cells mediate killing certain tumor cells and virally infected cells in non-MHC restricted manners. Additionally, NK cells are involved in the rejection of donor cells by bone marrow transplant recipients. Since LDCAM increases NK cell numbers, LDCAM, soluble LDCAM, or LDCAM fragments are useful in combating virally infected cells and infectious diseases. Similarly, LDCAM, soluble LDCAM, and LDCAM fragments are useful for killing tumor cells. Accordingly, within the scope of the present invention are methods for treating infectious diseases and methods for treating individuals afflicted with tumors. Such therapeutic methods involve administering LDCAM, soluble forms of LDCAM, or LDCAM fragments to an individual in need of increasing their numbers of NK cells in order to kill tumor cells or enhance their ability to combat infectious disease. Similarly, the therapeutic methods of the present invention can be carried out by administering LDCAM, soluble LDCAM, e.g. LDCAM fusion protein, or LDCAM fragments sequentially or concurrently in combination cytokines. Such cytokines include, but are not limited to, interleukins ("ILs") IL-15, IL-3 and IL-4, a colony stimulating factor ("CSF") selected from the group consisting of granulocyte macrophage colony stimulating factor ("GM-CSF") or GM-CSF/IL-3 fusions, or other cytokines such as TNF-α, CD40 binding proteins (e.g. CD40-L), 4-1BB antagonists (e.g. antibodies immunoreactive with 4-1BB and 4-1BB-L) or c-kit ligand.

Paragraph at page 15, lines 23-27:

Further within the scope of the present invention are methods for preventing or decreasing the effect of organ and ~~bond~~bone marrow transplant rejection by recipients of the transplant. Such methods involve treating recipients with a composition that includes a LDCAM inhibitor, thus inhibiting increases in NK cell populations and decreasing the ability of NK cells to reject transplants.

Paragraph at page 20, lines 9-16:

It is possible to utilize an affinity column comprising the ligand-binding domain of a LDCAM binding protein to affinity-purify expressed LDCAM polypeptides. LDCAM polypeptides can be removed from an affinity column using conventional techniques, e.g., in a high salt elution buffer and then dialyzed into a lower salt buffer for use or by changing pH or other components depending on the affinity matrix utilized. Alternatively, the affinity column may comprise an antibody that binds LDCAM. Example 10~~5~~ describes a procedure for employing LDCAM of the invention to generate monoclonal antibodies directed against LDCAM.

Paragraph at page 27, lines 6-12:

Following detection of an appropriate antibody titer, positive animals are provided one last intravenous injection of LDCAM~~B7L-1~~ in saline. Three to four days later, the animals are sacrificed, spleen cells harvested, and spleen cells are fused to a murine myeloma cell line, e.g., NS1 or preferably P3x63Ag8.653 (ATCC CRL 1580). Fusions generate hybridoma cells, which are plated in multiple microtiter plates in a HAT (hypoxanthine, aminopterin and thymidine) selective medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

Paragraph at page 27, lines 13-23:

The hybridoma cells are screened by ELISA for reactivity against purified B7L-1 by adaptations of the techniques disclosed in Engvall et al., *Immunochem.* 8:871, 1971 and in U.S. Patent 4,703,004. A preferred screening technique is the antibody capture technique described in Beckmann et al., (*J. Immunol.* 144:4212, 1990) Positive hybridoma cells can be injected intraperitoneally into syngeneic BALB/c mice to produce ascites containing high concentrations of anti-LDCAM~~B7L-1 L~~ monoclonal antibodies. Alternatively, hybridoma cells can be grown *in vitro* in flasks or roller bottles by various techniques. Monoclonal antibodies produced in mouse ascites can be purified by ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can also be used, as can affinity chromatography based upon binding to B7L-1.